THE MEASUREMENT OF ANTAGONISM

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(WITH THREE FIGURES)

Attention has been called to the need of a quantitative criterion of antagonism, and it has been shown that such a criterion is easily secured by mixing equally toxic solutions. In this paper[1] mixtures of more than two components were not considered. In order to clear up more fully the confusion which still exists in regard to the measurement of antagonism, it seems advisable to point out the special advantages of this method for mixtures containing three or more components.

The chief advantages lie in the fact that when equally toxic solutions are mixed, the additive effect remains unaltered, no matter how many components are used.[2] (By the additive effect is meant the effect produced when each salt acts independently of every other, when its toxicity is neither augmented nor diminished by the presence of other salts.) If antagonism exists, there will be better growth in the mixtures than in the pure solutions. The increase of growth over what would be expected if the effect were purely additive is the best measure of antagonism. It is best expressed as percentage of the additive effect.[1]

The determination of the additive effect is of first importance for quantitative results. The best method is that which permits us to make this determination most readily and exactly. As has been said, the method of mixing equally toxic solutions makes this determination simple and accurate, no matter how many components are used. Other methods permit this determination for binary solutions, but they involve much more labor, and as the number of components increases, the difficulty of determining the

[1] OSTERHOUT, W. J. V., Quantitative criteria of antagonism. BOT. GAZ. 58:178. 1914.

[2] When the toxic effect depends on ions, it may increase somewhat as the result of mixing the solutions because the ionization is increased. But this effect is usually small and it may be calculated without difficulty.

additive effect increases very rapidly. These methods usually consist in mixing unequally toxic solutions or in keeping the concentration of some salt or salts constant while varying that of the others.

The method of mixing equally toxic solutions also has a great advantage when the results are to be expressed graphically. As an illustration of this we may take mixtures of $NaCl+KCl+CaCl_2$. In the case of wheat it was found that the roots grew equally well in solutions of NaCl 0.12 M, KCl 0.13 M, and $CaCl_2$ 0.164 M. Mixtures of these solutions were prepared and the growth of the roots in these mixtures was measured after a period of 30 days. In order to show the results graphically, the composition of the solutions may be conveniently expressed by means of a triangular diagram as drawn in fig. 1.[3]

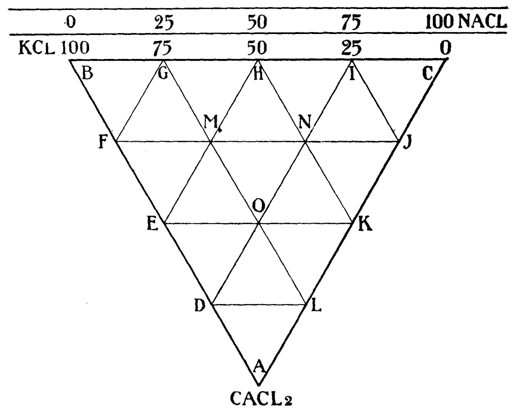


FIG. 1.—Diagram representing the composition of various mixtures of $KCl+NaCl+CaCl_2$: this serves as the base of the solid model shown in fig. 2.

The diagram consists of an equilateral triangle, the apices of which represent equally toxic pure solutions. Thus the point *A* represents pure $CaCl_2$ (0.164 M), *B* represents pure KCl (0.13 M), and *C* represents pure NaCl (0.12 M). All points on the sides of the triangle represent mixtures of two solutions only, the composition depending on the position of the point. Thus the point *H* represents a solution made by mixing the equally toxic solutions NaCl 0.12 M and KCl 0.13 M in such proportions that in the mixture 50 per cent of the dissolved molecules are NaCl and 50 per cent are

[3] These diagrams are employed in physical chemistry and have been used by SCHREINER and SKINNER in plant physiology (BOT. GAZ. 50:1. 1910). Finely ruled diagrams may be purchased from the Cornell Cooperative Society.

KCl. In the same way *G* represents a solution in which the molecular proportions are NaCl 25 per cent+KCl 75 per cent; *I* represents NaCl 75 per cent+KCl 25 per cent; *E* represents KCl 50 per cent+$CaCl_2$ 50 per cent; *K* represents NaCl 50 per cent+ $CaCl_2$ 50 per cent.

All points in the interior of the triangle represent mixtures of the three equally toxic solutions NaCl 0.12 M, KCl 0.13 M, and $CaCl_2$ 0.164 M. Along the line *FJ* are represented mixtures in which the dissolved molecules are 25 per cent $CaCl_2$; the line *EK* represents mixtures in which the dissolved molecules are 50 per cent $CaCl_2$; the line *DL* mixtures in which the dissolved molecules are 75 per cent $CaCl_2$. In the same way *FG* means 75 per cent KCl; *EH*, 50 per cent KCl; *DI*, 25 per cent KCl; *GL*, 25 per cent NaCl; *HK*, 50 per cent NaCl; and *IJ*, 75 per cent NaCl.

The point *M* is on the line *FJ*, meaning 25 per cent $CaCl_2$; it is also on the line *EH*, meaning 50 per cent KCl; and likewise on the line *GL*, meaning 25 per cent NaCl. It therefore represents a mixture of the three equally toxic solutions, NaCl 0.12 M, KCl 0.13 M, and $CaCl_2$ 0.164 M, in which the dissolved molecules are 25 per cent $CaCl_2$+50 per cent KCl+25 per cent NaCl. In the same way the point *O* represents a mixture in which the dissolved molecules are 50 per cent $CaCl_2$+25 per cent KCl+25 per cent NaCl.

It is obvious that the composition of any solution can be represented by selecting a suitable point on the diagram. At any such point an ordinate may be erected expressing the growth of the plant in that solution. When this has been done for a sufficient number of points, a solid model may be constructed which gives a complete description of the growth of the plant in all the solutions. Such a model is shown in fig. 2. The ordinates represent the aggregate length of roots per plant of wheat at the end of 30 days. The ordinates in the pure solutions are all equal (55 mm.), showing that the solutions are equally toxic. The ordinates were in part determined directly by experiment and in part calculated from data obtained by growing plants in solutions of approximately the same composition as those represented.[4]

[4] The data from which this model was constructed will appear in a subsequent publication.

From such a model the antagonism in any solution may be determined at once by measuring with calipers the height of the ordinate at the required point, subtracting 55 which is the amount of growth in the pure solutions, and in this case (since all the pure solutions are equally toxic) the amount of growth which would occur if the toxic actions of the salts were additive (that is, if each salt exerted its own toxic effect independently of the other salts); the result should then be divided by 55.[5]

In this case the additive effect is represented by a plane surface parallel to the plane which forms the base of the model. The height of this plane is indicated by the shading in the figure.

Other methods (as mixing unequally toxic solutions or keeping the concentration of one salt constant while varying that of the others) will give for the additive effect a curved surface very difficult to determine and not easily represented or measured on the model.

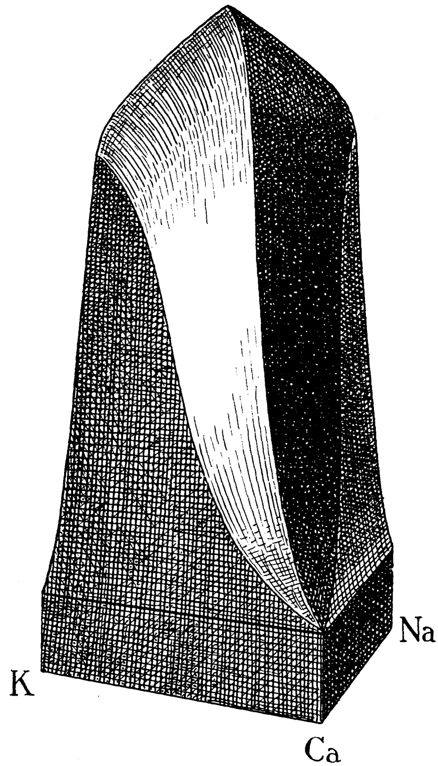
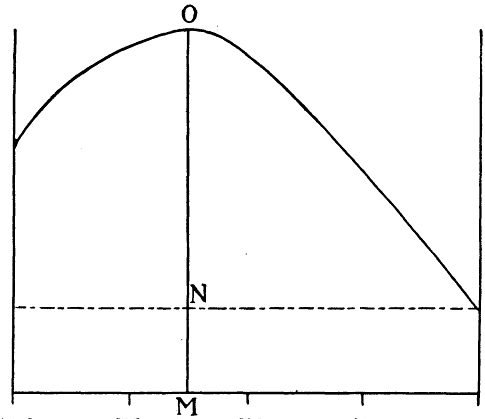


FIG. 2.—Solid model showing the forms of the antagonism curves in all possible mixtures of NaCl 0.12 M, KCl 0.13 M, and $CaCl_2$ 0.164 M.

With solutions of more than three components the results cannot be expressed in a solid model; but a graphical expression may easily be obtained in the following way. Let us suppose that equally toxic solutions of *A*, *B*, *C*, and *D* are to be mixed. A mixture of the first three may be made and called solution 1 (different mixtures may be called solution 2, etc.). To solution 1 various amounts of *D*

[5] For a fuller discussion see BOT. GAZ. **58**:178. 1914.

may be added and the results plotted as shown in fig. 3, in which the additive effect is expressed by the dotted line and the growth in the mixtures by the unbroken line. Antagonism at any point may be easily expressed. For example, the antagonism at the point M is $\frac{MO-MN}{MN}$.



Sol. I	100	75	50	25	0 %
D	0	25	50	75	100 %

FIG. 3.—Method of expressing antagonism in mixtures containing more than three components: three of the components (A, B, and C) are combined into solution 1 and various amounts of the fourth component (D) are added; the ordinates represent growth; the abscissas represent the composition of the mixtures; thus at the point M the mixture contains 62.5 cc. of solution 1 to each 37.5 cc. of solution D, the antagonism at M is $\frac{ON}{MN}$.

By the method of mixing unequally toxic, pure solutions or by the method of keeping the concentration of one salt constant while varying that of the others, the dotted line would become a curved one.

Summary

The measurement of antagonism in solutions containing more than two components presents no difficulty as long as we pursue the method of mixing equally toxic pure solutions.

Methods are suggested for the graphical expression of antagonism in mixtures of three or more components.

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